NTP Associate Director Dr. John Bucher welcomed everyone to the meeting, and thanked the SACATM members for the preparatory work they had done and the work they would do during the meeting. He thanked Dr. Niemi for chairing the meeting.

Dr. Kulpa-Eddy expressed her appreciation to the SACATM members for the considerable time and effort they put in to the meeting, and said she and the other ICCVAM members were looking forward to their comments and recommendations. Designated Federal Officer Dr. Lori White read the conflict of interest statement for SACATM.

IV. NICEATM-ICCVAM Update

NICEATM Director and ICCVAM Executive Director Dr. William Stokes updated SACATM on recent ICCVAM and NICEATM activities and priorities. He thanked the SACATM members for their participation, as well as the other stakeholders present at the meeting and joining by webcast. He noted the contributions of the ICCVAM representatives from 15 different Federal agencies, along with the scientists from these agencies who participate in the eight currently active ICCVAM interagency working groups.

Dr. Stokes' report included brief references to several items on the agenda for individual presentations later in the meeting.

- Endocrine Disruptor Chemical Screening Methods: The international validation study coordinated by NICEATM on the LUMI-CELL® stably-transfected transcriptional activation assay, which uses human ovarian carcinoma cells, was completed in 2010, followed by an International Peer Review meeting in March 2011. International validation of the MCF-7 Cell Proliferation Assay from CertiChem, Inc. was completed in March 2011; data are currently being analyzed and a review is expected later in 2011. The work is being coordinated by the ICCVAM Interagency Endocrine Disruptor Working Group, with liaisons from the ECVAM, JaCVAM, and KoCVAM.

- NICEATM-ICCVAM International Workshop on Vaccine Potency and Safety Testing: The workshop was held September 14-16, 2010. Nearly 200 scientists from 13 countries attended the meeting, which was co-organized with Health Canada, ECVAM, and JaCVAM. It addressed both human and veterinary vaccines. Proceedings will be published in *Procedia in Vaccinology* later in 2011.

- International Workshop on Alternative Methods for Rabies Vaccine Potency Testing: This workshop, to be held at the USDA National Centers for Animal Health in Ames, Iowa, October 11-13, 2011, is currently being organized by NICEATM-ICCVAM with ICATM partners. It will address alternative methods for both human and veterinary rabies vaccines, with attendees to include international scientific experts, regulatory authorities, and industry representatives.

- Allergic Contact Dermatitis (ACD) Safety Assessment Methods: ICCVAM Evaluations: Evaluations of several alternative methods for ACD safety assessments have been completed, the evaluation reports have been submitted, and the recommendations have been accepted and endorsed by both national and regulatory authorities. The evaluation report on the usefulness

of the LLNA for potency categorization has been completed and has been forwarded to the Secretary HHS for transmittal to Federal agencies.

• ACD Safety Assessment Methods: Other ICATM Collaborations: ICCVAM and NICEATM are working with ICATM partners to validate several other *in vitro* and *in chemico* methods. ECVAM will also be conducting a peer review of KeratinoSens, a promising new ACD test method. In the fall of 2011, JaCVAM will begin a validation study of another *in vitro* skin sensitization assay that uses a human monocyte cell line.

• Ocular Safety Testing Methods: ICCVAM Evaluation and Recommendations: Evaluation reports have been completed and recommendations were forwarded to the agencies through the Secretary of HHS in September 2010. Recommendations were provided for ten different alternative test methods and strategies.

• Ocular Safety Testing Methods: Other International and ICATM Activities: A proposal to update the OECD ocular test guideline to include additional humane endpoints and routine use of analgesics and anesthetics has been submitted and was circulated to member countries for comments in June 2011. Adoption of a guidance document on the use of histopathology in ocular safety is expected by summer 2011. Several other methods are currently undergoing validation studies. Also, a replacement ocular battery (RoBatt) using existing *in vitro* test methods for eye injury assessment is being developed under the National Institutes of Health-Food and Drug Administration (NIH-FDA) Regulatory Science Grant program.

• Ocular Safety Testing: Using Fewer Animals to Identify Chemical Eye Hazards: Based on a request from CPSC, ICCVAM developed proposed criteria for hazard classification using a 3-animal test that would provide equivalent hazard classifcation as the current requirements that use 6 to 18 animals. The results of an analysis of 481 eye safety tests indicate that a criterion of 1 or more positive animals in a 3 animal test would provide the same or greater level of eye hazard labeling as current requirements. The new criterion will allow the 3-animal test to be used, which will reduce animal use by 50-83% compared to the current requirements. ICCVAM recommendations are currently in progress.

• Acute Systemic Toxicity Activities: NICEATM-ICCVAM is collaborating with ECVAM to develop *in vitro* models for human hepatic metabolism and toxicity. An acute dermal up-and-down procedure is also under development. The 3T3 neutral red uptake cytotoxicity test is under evaluation by ECVAM to determine if it can be used to classify “non-toxic” substances in the European Union (EU) without animal testing.

• Genetic Toxicity Test Method Activities: JaCVAM is leading international validation studies for *in vivo* and *in vitro* Comet assays. JaCVAM and ECVAM have made considerable progress on four types of cell transformation assays. The ICCVAM Interagency Genetic Toxicity Working Group and ICATM liaison have contributed to these efforts.

• Recent Test Method Nominations: New nominations include an *in vitro* pyrogen test method for assessing non-endotoxin pyrogens, and *in vitro* assays to detect and quantify botulinum neurotoxins.

• Developing Future Test Methods: High throughput *in vitro* screening is taking place in the Tox21 collaboration, using the NIH Chemical Genomics Center's new robotic facility to screen 10,000 chemicals in an effort to identify toxicity pathways. NICEATM nominated over 900 chemicals for inclusion in the screening initiative, and recently nominated a nuclear receptor assay to use in the screening effort. The EPA's ToxCast™ program is also using 600 *in vitro* assays on a smaller subset of chemicals. NICEATM-ICCVAM will monitor the results of those studies for *in vitro* test methods with pathway-based predictive biomarkers.

• Outreach Activities: Recent NICEATM-ICCVAM outreach activities have included two workshops on best practices for regulatory safety testing, numerous posters, and an informational session on ICATM at the 2011 Society of Toxicology meeting. NICEATM-ICCVAM will have 11 presentations at the Eighth World Congress on Alternatives and Animal Use in the Life Sciences in Montreal in August.

Dr. Stokes concluded the presentation with a brief update on ICATM including the modification signed March 8, 2011 to add KoCVAM to ICATM.

V. Regulatory Acceptance of ICCVAM-Recommended Alternative Test Methods

Dr. Stokes provided SACATM with an update on regulatory acceptance of ICCVAM-recommended alternative test methods, both in the U.S. and internationally.

• *LLNA for ACD*: ICCVAM recommendations for two non-radioactive LLNA versions and for an expanded applicability domain of the LLNA were transmitted to Federal agencies on June 12, 2010. He described several 3Rs-related advantages of the LLNA, which was first recommended by ICCVAM in 1999 as an alternative to the traditional guinea pig test method. In terms of reduction in animal use, the 2009-updated ICCVAM LLNA protocol uses 20 animals per test, versus a minimum of 30 in the guinea pig protocol and 25 in the original LLNA (a 20% reduction). Regarding refinement, the LLNA avoids the pain and distress associated with guinea pig tests since it does not involve the elicitation phase of ACD. ICCVAM previously recommended a reduced LLNA (rLLNA) for substances deemed unlikely to cause an ACD response, which uses just 12 animals per test, a 40% reduction compared to the standard assay. With regard to the two non-radioactive LLNA versions and the expanded applicability domain, agencies concurred with ICCVAM recommendations where applicable to their agency, although the FDA did note limitations of the LLNA- Daicel Adenosine Triphosphate (DA) assay, including a potential for false positives in the weakly positive response range. The new and updated LLNA-based test methods have been formally adopted and published by the OECD.

Ocular safey testing: Federal agencies recently indicated their acceptance of ICCVAM recommendations for the routine use of analgesics, topical anesthetics, and humane endpoints for required *in vivo* ocular safety testing. The OECD proposal for incorporation of these recommendations in the international guidelines is under consideration and is expected for adoption in 2012. ICCVAM has recommended the use of the CM *in vitro* method to identify substances not requiring ocular hazard labeling. ICCVAM reviewed the Bovine Corneal Opacity